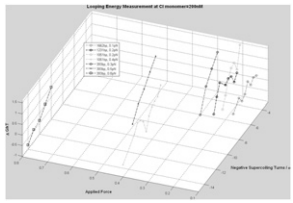
different locations along the genome and changes during the cell cycle, little is known about how localized changes in DNA supercoiling, under enzyme-generated DNA tension, perturb critical protein-DNA interactions. Here we investigated how DNA supercoiling affects stability of the lambda repressor-mediated DNA loop that acts as a genetic switch between lysogeny (quiescence) and lysis (virulence).

We performed single molecule magnetic tweezers measurements to record lambda repressor-mediated DNA loop formation and breakdown and to measure the stability of the loop as a function of negative supercoiling, loop size and DNA tension at physiological repressor concentration. The level of negative supercoiling required for loop formation increases with loop size and that, in general, negative supercoiling stabilizes the loop. Since genomic supercoiling depends on the energy level of the cell which is tightly associated with its health status, we propose that the switch to lysis is favored by the destabilization of the lambda repressor-mediated loop that follows loss of DNA supercoiling in suffering cells.



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Energetic and Structural Bases of DNA Overstretching Transition and its Cooperativity

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By using a dual laser optical tweezers with a fast force feedback, we were able to record the exponential elongation following force steps imposed on Δ-phage ds-DNA molecule and on two ~3000 bp segments of the molecule, with either high (59%) or low (46%) CG content. The rate of elongation (r) following a 2pN step imposed on the whole molecule in the region of the overstretching transition varies with the force in a U shaped way, while its corresponding elongation varies in a reverse-U shaped way (Bianco et al., Biophys. J. 101, 866-874, 2011). The minimum of the r - force relation (rmin) and the corresponding maximum elongation (ΔLe) do not change significantly with temperature in the range 25-10°C (mean values 4.9 ± 0.2 s-1 and 3.9 ± 0.2 μm respectively) and are shifted progressively to higher forces at lower temperature. The load- and temperature-dependence of the elongation rate supports the two state (B-S) nature of the transition, yielding a cooperativity of 22 bp and revealing the absence of an enthalpic contribution to the transition free energy barrier.

At room temperature the AT rich segment shows large hysteresis on relaxation, implying a predominance of melting on overstretching, while the CG rich segment does not show hysteresis. At temperature below 10°C hysteresis completely disappears for both segments. rmin and ΔLe are 3.9 ±1.4 s-1 and 0.3 ± 0.1 nm for the CG and 4.3 ± 1.3 s-1 and 0.3 ± 0.1 nm for the AT. Fitting the data with the two state reaction model shows a cooperativity of ~ 15 and 32 for the AT and CG respectively, which correlates with the average distance between groups of more than four consecutive A or T bases.

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Sequence Dependence of the "B-to-S" DNA Overstretching Transition

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Torsion unconstrained double-stranded DNA can undergo an ''overstretching'' transition at forces around 65 pN, which elongates the DNA backbone by about 1.7-fold. Recent experiments have revealed two distinct structural transitions during DNA overstretching: a ''peeling'' of one strand from the other leads to a single-stranded DNA, and a ''B-to-S'' transition to a mysterious double-stranded ''S-DNA''. The peeling transition has been determined to depend on DNA sequence. The knowledge of sequence dependence of the "B-to-S" transition, however, is still lacking. Here, we report for the first time that, the GC-rich DNA requires a slight higher transition force compare to the AT-rich DNA. This finding helps us to understand the structure of the mysterious double-stranded S-DNA.

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Revealing the Competition between Peeled-Ssdna, Melting Bubbles and S-DNA during DNA Overstretching using Fluorescence Microscopy

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Understanding the structural changes occurring in double-stranded (ds)DNA during mechanical strain is essential to build a quantitative picture of how proteins interact and modify DNA. However, the elastic response of dsDNA to tension is only well-understood for forces < 65 pN. Above this force, torsionally unconstrained dsDNA gains ~70% of its contour length, a process known as overstretching. The structure of overstretched DNA has proved elusive, resulting in a rich and controversial debate in recent years. At the centre of the debate is the question of whether overstretching yields a base-paired elongated structure, known as S-DNA, or instead forms single-stranded (ss)DNA via base-pair cleavage. Here, we show clearly, using a combination of fluorescence microscopy and optical tweezers, that both S-DNA and base-pair melted structures can exist, often concurrently, during overstretching. The balance between the two models is affected strongly by temperature and ionic strength. Moreover, we reveal, for the first time, that base-pair melting can proceed via two entirely different processes: progressive strand unpeeling from a free end in the backbone, or by the formation of 'bubbles' of ssDNA, nucleating initially in AT-rich regions. We demonstrate that the mechanism of base-pair melting is governed by DNA topology: strand unpeeling is favored when there are free ends in the DNA backbone. Our studies settle a long running debate, and unite the contradictory dogmas of DNA overstretching. These findings have important implications for both medical and biological sciences. Force-induced melting transitions (yielding either peeled-ssDNA or melting bubbles) may play active roles in DNA replication and damage repair. Further, the ability to switch easily from DNA containing melting bubbles to S-DNA may be particularly advantageous in the cell, for instance during the formation of RNA within transcription bubbles.

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Determining the Elasticity of Short DNA Fragments using Optical Tweezers and Protein-Mediated DNA Loop Formation Assays

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In the light of the recent controversy over whether DNA on short length scales becomes more elastic or not, we contribute two additional measurements that take sequence effects into account: We have directly stretched short DNA molecules of varying AT / CG content with optical tweezers to determine their persistence length. Scaling the result for known end-point entropic effects, we find a persistence length of 44 ± 2nm and 59 ± 3nm for the AT and CG rich constructs, respectively, but no appreciable overall softening, in line with our previous measurements that found no softening in DNA molecules as short as 250 bp. Secondly, we have used tethered particle motion (TPM) microscopy to observe protein-mediated DNA loop formation in the lactose repressor system with the same DNA constructs between the operators. While an absolute determination of the persistence length of the DNA from such kinetic measurements is notoriously difficult, as ongoing discussions of ring cyclization experiments by various groups suggest, we can unequivocally state that any sequence effect in these measurements is much smaller than what we found in the stretching experiments with the optical tweezers. This suggests two distinct response regimes of DNA to force: one for low curvatures commensurate with thermal forces, in which elastic-rod descriptions for the DNA hold and sequence effects are observed, and a second non-linear high curvature region in which elasticity is not sequence-dependent and likely dominated by the conformation of the phosphate backbone.

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Constructing an Energy Landscape for the Hybridization of Short Oligonucleotides

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The hybridization of short oligonucleotides plays a critical role in many biological systems, from DNA replication to gene silencing. Despite extensive studies, details on the mechanism of this process on the shortest length scale (~10 bp) remain poorly understood. We use high-resolution optical tweezers with simultaneous fluorescence microscopy to investigate the hybridization of single oligonucleotides under tension[1]. We measure the change in end-to-end extension upon annealing and melting as well as the unbinding kinetics of short (7-12 bp), fluorescently labeled oligonucleotides hybridizing to a complementary DNA sequence tethered between trapped beads. Our results allow us to construct an energy landscape of oligonucleotide hybridization along well-defined reaction coordinates. Interestingly, our measurement of the distance to the transition state for DNA melting as a function of oligonucleotide length suggests that the extension of the transition state matches that of a state with 6 bp hybridized. Lastly, we find that the change in extension upon hybridization deviates from extensible-wormlike-chain behavior at forces >10 pN. We discuss possible models to explain these results.

1. M. J. Comstock, Taekjip Ha, and Y. R. Chemla, Nat. Meth. **8**, 4 (2011).